Experiment Design for reconstruction of pTAL1 in GGA

DAY1

1. Choose proper promoter, RBS and terminator for the vector.
Promoter and RBS:

(1) Constitutive

BBa_K081005: http://parts.igem.org/Part:BBa_K081005

Spring 2014 Distribution 16E 201

(2) Inducible

BBa_K081005+**BBa_C0040**+BBa_B0015+BBa_K081005

BBa_C0040: http://parts.igem.org/wiki/index.php?title=Part:BBa_C0040

Spring 2014 Distribution 2P

Terminator:

BBa_B0015: http://parts.igem.org/wiki/index.php?title=Part:BBa_B0015

Spring 2014 Distribution 3F 2014

TetR repressible promoter
BBa_R0040:http://parts.igem.org/Part:BBa_R0040
Spring 2014 Distribution 6F 201
2. Assemble method: Assembly standard 10
http://parts.igem.org/Assembly_standard_10

3. Transform the selected plasmids and plate them onto specific antibiotics-containing plate.
4. Design primers for the wanted part of pTAL.
Forward:

IG14003-pTAL-for:
ATTGAATTCGCGGCCGCTTCTAGATGGATCCCATTCGTCCGC

Reverse:

IG14002-pTAL-re:

TATCTGCAGCGGCCGCTACTAGTATTTCACTGAGGCAATAGCT

DAY2

1. Pick up several colonies from the plates and incubate them for 6 hours.
2. Extract plasmids and run a gel or sequence it for further confirmation (day3).

Enzyme digestion system:

plasmids 10μl

ddwater 7μl

CutSmart buffer 2μl

EcoRI 0.5μl For B0015, use EcoRII and Esp3I

SpeI 0.5μl For K081005, use NheI and Esp3I

Incubate in 37°C for 2h.

Expected results: K081005: 0.6k+0.4k; B0015: 0.6k + 1.5k

3. After getting the synthesized oligo for the primers, use them to get PCR products of pTAL.

The PCR reaction system (50μl):

pTAL 1ul (20ng)

dNTP 1ul (10mM)

Primer1 (IG14003-pTAL-for) 2.5ul (10nM)

Primer2 (IG14003-pTAL-for) 2.5ul (10nM)

Phusion Polymerase 0.5μl

Phusion Reaction Buffer (HF & GC) 10μl

(another 1.5ul DMSO in GC buffer)

ddwater 32.5ul

Reaction protocol:

(1) 95°C 4min

(2) 95°C 30s

(3) 55°C 30s

(4) 72°C 90s

(5) Goto Step 2 for 30 cycles

(6) 72°C 10min

(7) 4°C Forever

Reference: https://www.neb.com/protocols/1/01/01/pcr-protocol-m0530

4. Run a gel for the PCR products (2k) and if the result is positive, sequence it.

DAY3

1. Use EcoRI and SpeI to digest the PCR products and EcoRI and XbaI to digest BBa_B0015.

Also, use EcoRI and SpeI to digest the PCR products and BBa_B0015 respectively.

Enzyme Digestion System:

PCR products 10μl/5μl

ddwater 7μl/12μl

CutSmart buffer 2μl

EcoRI 0.5μl

SpeI/XbaI 0.5μl

Incubate in 37°C for 2h.

2. Use DNA clean kit to pure the digestion product.
3. Ligation:

PCR products: backbone = 3:1

Quick ligase buffer 5μl

Quick ligase 0.5μl

ddwater

Incubate at 37°C for 5min.

4. Transformation and Screening (Chl-antibiotics)
5. Use the same method to ligate BBa_C0040 and BBa_B0015.

DAY4

1. Pick up several colonies and culture them in chl-containing culture.

DAY5

1. Extract the reconstructed plasmids (TAL + terminator).
2. Enzyme digestion and run a gel.

Enzyme Digestion System:

plasmids 5μl

ddwater 12μl

CutSmart buffer 2μl

EcoRI 0.5μl

SpeI 0.5μl

Incubate at 37°C for 2h.

Expected result: 1k: 3k.

3. Sequence it.

DAY6

If the result is positive, continue to construct the plasmid.

1. Use EcoRI and SpeI to digest the ligated products and EcoRI and XbaI to digest BBa_Q04400/BBa_K081005.

2. Run a gel to separate wanted bands from digestion products (TAL + terminator.)
3. Use DNA clean kit to pure the digestion product of Use DNA clean kit to pure the digestion product.
4. Ligation.

PCR products: backbone = 3:1

Quick ligase buffer 5μl

Quick ligase 0.5μl

ddwater

Incubate at 37°C for 5min.

5. Transformation and Screening (for K081005, chl)
6. Use the same method to ligate BBa_C0040+BBa_B0015 and BBa_K081005).

DAY7

1. Extract the plasmids.
2. Enzyme digestion and run a gel.

Enzyme Digestion System:

plasmids 5μl

ddwater 12μl

CutSmart buffer 2μl

EcoRI 0.5μl

SpeI 0.5μl

Incubate at 37°C for 2h.

Expected results: 2k: 3k.

3. Sequence it.